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Research Note

Movement and Persistence of *Salmonella* in Broiler Chickens following Oral or Intracloacal Inoculation

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ABSTRACT

The dissemination of *Salmonella* into various lymphoid-like organs in young broiler chicks after oral and intracloacal inoculation was studied. A three-strain cocktail of *Salmonella* Typhimurium, *Salmonella* Montevideo, and *Salmonella* Enteritidis was administered either orally or intracloacally to day-old chicks. After 1 h, 1 day, or 1 week, the ceca, thymus, liver and gallbladder, spleen, and bursa were sampled for the presence of *Salmonella.* There was a marked difference in the recovery of *Salmonella* 1 h postinoculation. Only 6 of 50 samples from orally inoculated chicks were positive compared with 33 of 50 samples from cloacally inoculated samples. In comparison, 24 h and 1 week after inoculation, there was no difference in the number of positive samples between oral or cloacal inoculation. The rapidity of the translocation of the *Salmonella* from the cloacal inoculum compared to the oral inoculum is likely due to the transient time required for *Salmonella* to move through the alimentary tract. The method of inoculation did not affect the distribution of serogroups. Of the three serotypes in the composite inoculum, the *Salmonella* Enteritidis (group D) was recovered only twice in replication 1 and not at all in replication 2. Both the *Salmonella* Typhimurium (serogroup B) and the *Salmonella* Montevideo (serogroup C1) were recovered extensively throughout the study.

Control of *Salmonella* in poultry is complicated because there are numerous potential sources of *Salmonella* contamination in an integrated poultry operation, including chicks, feed, rodents, wild birds, insects, transportation, the farm environment, and the processing plant environment. Many factors can influence the relative importance of various sources of *Salmonella*; these include (i) age of the chicken, (ii) survival through the gastric barrier, (iii) competing bacteria in the intestinal tract, (iv) availability of a hospitable colonization site, (v) nature of diet, (vi) physiological status of the chicken, (vii) health and disease status of the chicken, and (viii) medication effects, which will influence the potential colonization of chickens with *Salmonella (1).*

The age of the chicken at the time of exposure to *Salmonella* has been documented to play a critical role in the colonization or infection of the chicken. Milner and Shaffer *(10)* first observed that colonization of chicks was dose dependent and varied with day of infection when chicks were challenged orally with 10 different serotypes of *Salmonella.* They found that day-old chicks could be infected with less than five cells of *Salmonella* and that later infection was irregular and took higher doses of *Salmonella* to achieve. Sadler et al. *(11)* also found the level of intestinal infection as evidenced by fecal shedding to be correlated with bird age and inoculum dose.

The route of exposure to *Salmonella* can also play a role in colonization. Cox and coworkers *(3)* demonstrated that about 100-fold fewer *Salmonella* Typhimurium were required to colonize young chicks by the intracloacal route than by oral gavage. It was hypothesized that the low pH of the upper gastrointestinal tract contributes to the higher levels of *Salmonella* required to colonize young chicks via the oral route. The production of *Salmonella*-colonized seeder birds was also demonstrated to occur when the *Salmonella* was introduced into any body opening of the chick, including the mouth, cloaca, eye, and naval *(2).*

The movement and potential localization of *Salmonella* once it gets into the chicken is not fully understood. Clearly, the intestinal tract becomes colonized with the *Salmonella.* There is additional evidence to suggest that the *Salmonella* may be translocated to other organs. *Salmonella* has been found to persist in the liver of orally inoculated laying hens for up to 22 weeks postinoculation *(5)* and to persist in the spleen for up to 40 weeks *(5, 13).* The objective of this study was to determine if the dissemination of *Salmonella* into various lymphoid-like organs occurs in young broiler chicks after oral and intracloacal inoculation and if it persists for at least a week.

MATERIALS AND METHODS

Bacterial cultures. Four strains of nalidixic acid–resistant *Salmonella* were used to inoculate chicks, *Salmonella* Typhimurium (1), *Salmonella* Montevideo (1), and *Salmonella* Enteritidis (2). All strains were maintained on Trypticase soy agar (Becton Dickinson [BD], Sparks, Md.) until needed. The cultures were streaked onto brilliant green sulfa (BGS; BD) agar plates containing 200 ppm of nalidixic acid (Nal; Sigma Chemical Co., St. Louis, Mo.). The plates were incubated overnight at 37°C. Frozen

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stock cultures were maintained at −80°C in brain heart infusion broth (BD) and 16% glycerol (Sigma).

Inoculum preparation. The cultures were removed from the incubator and a suspension was made with sterile 0.85% saline. The absorbance value was adjusted to 0.2 at 540 nm. This gave approximately 1×10^8 cells per ml. Equal volumes of each culture were added to one tube and the inoculum was enumerated onto BGS with Nal agar plates.

Animals and handling. For each of two replications, 60 day-of-hatch chicks were purchased from a local hatchery. The chicks were transported to the laboratory in a reusable chick transport tray that had been sterilized with an approved disinfectant. At the laboratory, they were inoculated orally ($n = 24$), cloacally ($n = 24$), or not at all (control birds, $n = 12$). Each treatment was divided into three equal groups, with one group of each treatment held in the laboratory for 1 h postgavage. The other two sets of treatment groups for each treatment were transported to chicken isolation facilities in Watkinsville, Ga., where they were housed in positive-pressure isolation units (Controlled Isolation Systems, Inc., San Diego, Calif.). The units contained individual dividers (made from corrugated cardboard) for each chick, jug drinkers, mesh flooring, and a pan feeder, and air exchange was provided by a filtered positive-pressure ventilation system. The chicks were provided water and feed ad libitum.

Sampling protocol. At 1 h, 1 day, and 1 week (treatment, $n = 5$; control, $n = 4$) postinoculation for each replication, the broiler chicks were humanely sacrificed by cervical dislocation. The exteriors of the chicks were sprayed with 70% ethanol and aseptically opened. The thymus, spleen, liver and gallbladder, and bursa of Fabricius were aseptically removed from both the treatment and control broiler chicks prior to removal of the ceca to reduce the possibility of fecal contamination of the organs. All samples were placed into sterile plastic sampling bags (Fisher Scientific, Pittsburgh, Pa.), labeled accordingly, and packed on ice.

Isolation procedure. All samples were individually weighed, and buffered peptone water three times the weight of the sample (Oxoid, Inc., Basingstoke, UK) was added to the bags. The bags were stomached (Techmar Company, Cincinnati, Ohio) for 60 s before being placed into the 37°C incubator for overnight preenrichment. After preenrichment, 0.1 ml of each sample was transferred to tetrathionate brilliant green broth Hajna (BD). After overnight enrichment at 42°C, 0.1 ml of the samples was transferred to Rappaport-Vassiliadas broth (BD). The samples were incubated overnight at 37°C and then streaked onto BGS, BGS agar with Nal, and modified lysine iron agar (Oxoid). The plates were incubated overnight at 37°C. Typical colonies were picked from the plates onto triple sugar iron agar (BD) and lysine iron agar slants. The slants were incubated overnight at 37°C with the caps loose. Slants yielding typical results were serogrouped and the data were recorded.

RESULTS AND DISCUSSION

Both in replication 1 (Table 1) and replication 2 (Table 2) *Salmonella* was recovered from uninoculated control chicks 24 h and 1 week after inoculation. All positive samples were detected on *Salmonella* plating media that did not contain nalidixic acid, indicating that the chicks had likely been exposed to *Salmonella* in the hatchery. Analysis of the serotyping data (Tables 1 and 2) shows that not only was *Salmonella* present in the chicks before inoculation, but there were multiple serogroups: C1, B, E, and A-I. There-

TABLE 1. Salmonella-*positive organs and corresponding serogroups isolated from juvenile chicks at 1 h, 24 h, and 1 week of growth after oral or cloacal inoculation (replicate 1)*[a]

Inoculation route	Time postinoculation	Thymus	Serogroup	Liver/ gallbladder	Serogroup	Spleen	Serogroup	Ceca	Serogroup	Bursa	Serogroup
Control	1 h	0/4[b]	N/A	0/4	N/A	0/4	N/A	0/4	N/A	0/4	N/A
Oral		3/5	B (1)[c], C1 (2)	0/5	N/A	0/5	N/A	1/5	B (1), C1 (1)	1/5	C1 (1)
Cloacal		1/5	C1 (1)	1/5	A-I (1), C1 (1)	2/5	A-I (1), C1 (2)	3/5	C1 (3)	4/5	A-I (1), C1 (4)
Control	24 h	0/4	N/A	0/4	N/A	0/4	N/A	0/4	N/A	0/4	N/A
Oral		1/5	C1	1/5	C1 (1)	0/5	N/A	5/5	A-I (1), B (1), C1 (4)	1/5	C1 (1), E (1)
Cloacal		3/5	A-I (2), B (1), C1 (2)	1/5	C1 (1)	2/5	A-I (1), C1 (2)	5/5	A-I (2), C1 (5)	5/5	A-I (1), C1 (5)
Control	1 wk	3/4[d]	B (1), C1 (2)	3/4[d]	B (1), C1 (2)	1/4[d]	C1 (1)	0/4	N/A	0/4	N/A
Oral		3/5	C1 (3)	5/5	A-I (1), C1 (5), D (1)	2/5	C1 (2)	5/5	A-I (1), C1 (5)	5/5	A-I (1), B (2), C1 (3), E (1)
Cloacal		1/5	C1 (1)	4/5	C1 (4)	3/5	A-I (1), C1 (2)	5/5	C1 (5), D (1)	5/5	A-I (1), C1 (5)

[a] Chicks were inoculated with approximately 10^6 cells each of *Salmonella* Typhimurium (serogroup B), *Salmonella* Enteritidis (serogroup D), and *Salmonella* Montevideo (serogroup C1). N/A, not applicable.

[b] No. positive/no. sampled.

[c] Number in parentheses represents the number of isolates within that particular serogroup. It should be noted that multiple serogroups were recovered from some samples.

[d] Naturally occurring *Salmonella* that were not nalidixic acid resistant were present.

TABLE 2. Salmonella-*positive organs and corresponding serogroups isolated from juvenile chicks at 1 h, 24 h, and 1 week of growth after oral or cloacal inoculation (replicate 2)*[a]

Inoculation route	Time postinoculation	Thymus	Serogroup	Liver/ gallbladder	Serogroup	Spleen	Serogroup	Ceca	Serogroup	Bursa	Serogroup
Control	1 h	0/4[b]	N/A	0/4	N/A	0/4	N/A	0/4	N/A	0/4	N/A
Oral		0/5	N/A	0/5	N/A	0/5	N/A	1/5	B (1), C1 (1)	0/5	N/A
Cloacal		4/5	B (2)[c], C1 (3)	5/5	B (3), C1 (3)	3/5	B (1), C1 (2)	5/5	B (2), C1 (5)	5/5	B (3), C1 (5)
Control	24 h	1/4[d]	C1 (1)	0/4	N/A	0/4	N/A	0/4	N/A	1/4[d]	B (1), C1 (1)
Oral		2/5	B (1), C1 (1)	1/5	B (1)	4/5	B (2), C1 (3)	5/5	B (4), C1 (4)	3/5	B (3), C1 (1)
Cloacal		2/5	B (2), C1 (2)	1/5	B (1)	1/5	B (1), C1 (1)	5/5	B (1), C1 (5)	5/5	B (3), C1 (4)
Control	1 wk	1/4[d]	B (1)	2/4[d]	B (2), C1 (2)	0/4	N/A	1/4[d]	B (1)	2/4[d]	B (2)
Oral		3/5	B (3), C1 (2)	5/5	B (5), C1 (1)	3/5	B (2), C1 (2)	5/5	B (4), C1 (2)	5/5	B (5), C1 (1)
Cloacal		3/5	B (2), C1 (2)	3/5	B (3)	2/5	B (2)	4/5	B (3), C1 (2)	4/5	B (2), C1 (4)

[a] Chicks were inoculated with approximately 10^6 cells each of *Salmonella* Typhimurium (serogroup B), *Salmonella* Enteritidis (serogroup D), and *Salmonella* Montevideo (serogroup C1). N/A, not applicable.

[b] No. positive/no. sampled.

[c] Number in parentheses represents the number of isolates within that particular serogroup. It should be noted that multiple serogroups were recovered from some samples.

[d] Naturally occurring *Salmonella* that were not nalidixic acid resistant were present.

fore, in addition to the known inoculum, chicks had been exposed naturally to *Salmonella.*

One hour postinoculation, *Salmonella* was not recovered from uninoculated control chicks but was recovered from 10, 20, and 30% of bursa, ceca, and thymus samples, respectively, after oral inoculation and from 50 to 90% of all organs after cloacal inoculation (Table 3). Twenty-four hours postinoculation, *Salmonella* was recovered from only 12.5% of thymus and bursa samples from uninoculated control chicks but was recovered from 20, 30, 40, 40, and 100% of the liver and gallbladder, thymus, spleen, bursa, and ceca samples, respectively, after oral inoculation and from 20, 30, 50, 100, and 100% of the liver and gallbladder, spleen, thymus, bursa, and ceca samples, respectively, after cloacal inoculation (Table 3). One week after inoculation, *Salmonella* was recovered from 12.5, 12.5, 25, 50, and 62.5% of spleen, ceca, bursa, thymus, and liver and gallbladder samples, respectively, from uninoculated control chicks compared with 50 to 100% of all organ samples from both oral and cloacal inoculation chicks (Table 3).

There was a marked difference in the recovery of *Salmonella* 1 h postinoculation. Only 6 of 50 samples from orally inoculated chicks were positive compared with 33 of 50 samples from cloacally inoculated samples (Table 3). In comparison, 24 h and 1 week after inoculation, there was no difference in the number of positive samples between oral or cloacal inoculation (Table 3). The rapidity of the translocation of the *Salmonella* from the cloacal inoculum compared to the oral inoculum is likely due to the transient time required for *Salmonella* to move through the alimentary tract. The method of inoculation did not affect the distribution of serogroups. Of the three serotypes in the composite inoculum, the *Salmonella* Enteritidis (group D) was recovered only twice in replication 1 and not at all in replication 2. Both the *Salmonella* Typhimurium (serogroup B) and the *Salmonella* Montevideo (serogroup C1) were recovered extensively throughout the study. The difference in recovery of these strains suggests that the strain of *Salmonella* Enteritidis used in this study did not compete well with the other serotypes. More strains would have to be compared before it could be concluded that this would be the same for all *Salmonella* Enteritidis strains.

This study has clearly demonstrated that *Salmonella* can be translocated to the lymphoid-like organs of the chicken by either oral or intracloacal exposure. The route or method of dissemination was not determined. Kimura et al. *(7)* had previously found that *Enterobacteriaceae,* streptococci, and lactobacilli colonize the bursa of Fabricius and the intestinal tract shortly after hatching, and bursal lymphocytes have been shown to migrate to peripheral lymphoid tissues, e.g., the spleen *(6). Campylobacter,* the other principal bacterial pathogen associated with chickens, has been demonstrated to translocate to the ceca and liver and/or gallbladder of chicks following oral inoculation *(12). Campylobacter jejuni* has been shown to propagate into the spleen, liver, and lungs of Japanese quails after they were experimentally inoculated orally *(8, 9).* Maruyama and Katsube *(8)* found *Campylobacter jejuni* persisted in the abovementioned organs for up to 17 days postinoculation. In a

TABLE 3. *Total* Salmonella*-positive organs from juvenile chicks at 1 h, 24 h, and 1 week of growth after oral or cloacal inoculation*[a]

Inoculation route	Time postinoculation	Thymus	Liver/ gallbladder	Spleen	Ceca	Bursa
Control	1 h	0/8[b]	0/8	0/8	0/8	0/8
Oral		3/10	0/10	0/10	2/10	1/10
Cloacal		5/10	6/10	5/10	8/10	9/10
Control	24 h	1/8[c]	0/8	0/8	0/8	1/8[c]
Oral		3/10	2/10	4/10	10/10	4/10
Cloacal		5/10	2/10	3/10	10/10	10/10
Control	1 wk	4/8[c]	5/8[c]	1/8[c]	1/8[c]	2/8[c]
Oral		6/10	10/10	5/10	10/10	10/10
Cloacal		4/10	7/10	5/10	9/10	9/10

[a] Chicks were inoculated with approximately 10^6 cells each of *Salmonella* Typhimurium (serogroup B), *Salmonella* Enteritidis (serogroup D), and *Salmonella* Montevideo (serogroup C1).
[b] No. positive/no. sampled.
[c] Naturally occurring *Salmonella* that were not nalidixic acid resistant were present.

related study, Maruyama and Katsube *(9)* found liver samples to be positive 19 and 20 days after oral inoculation. In a 1973 study, the examination of the bursa of Fabricius showed that bacteria were always present in considerable numbers, but the involvement of the bursa of Fabricius in translocation was not established *(4)*.

Regardless of the route of exposure to *Salmonella*, all tested lymphoid-like organs in the chicken were colonized with *Salmonella*. When the exposure was via the cloacae, the spread of the *Salmonella* throughout the body was more rapid, suggesting the possible involvement of bursal lymphocytes. Further studies are needed to elucidate the mechanisms of dissemination of the *Salmonella* through the body and into the lymphoid-like organs.

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